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# The Effect of Aerobic exercise training on Mitochondrial Dynamics in the Muscle Tissue of Diabetic Rats

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## Abstract

**Background**: Dysfunction of Skeletal muscle mitochondrial is often associated with some of the metabolic diseases for instance diabetes.

**Aim:** the aim of this research was to study the effect of eight weeks of aerobic exercise training on proteins levels of MFN1, MFN2, FIS1 and Drp1 in gastrocnemius muscle of diabetic rats.

**Martials and Methods:** In the present study, the 20 male Wistar rats (age: 8 weeks, weight: 200±20 g) were randomly divided into four groups of Diabetic Control (DC), Diabetic Training(DT), Healthy Control (HC) and Healthy Training (HT). Induction of type-2 diabetes was done by combining of High-Fat Diet and Streptozotocin (30 mg/kg) injection . In the training groups, the aerobic exercise training program was carried out for eight weeks based on the principle of gradual overload, and the training intensity was increased from 15 m/min for 30 minutes in the first week to 25 m/min for 60 minutes in the last week. 48 hours after the last training session, the rats were unconscious, then the tissue of the gastrocnemius muscle was extracted and kept at -80 temperature. The levels of all proteins were evaluated by western blot technique. One-way ANOVA analysis with Tukey's post hoc was used for comparison of variables in between groups at a significance level of 0.05 by SPSS-23 software.

**Results:** Induced diabetes caused by the combination of high-fat diet and streptozotocin injection led to a significant decrease in MFN1 (P=0.001) and MFN2 (P=0.019) proteins and a significant increase in FIS1(P=0.012) and Drp1(P=0.023) proteins in the gastrocnemius muscle of diabetic rats. However, aerobic exercise training led to a significant increase in MFN1(P=0.009) and MFN2 (P=0.015) and a significant decrease in FIS1(P=0.002) and Drp1(P=0.034) proteins in the muscle of diabetic rats (P< 0.05).

**Conclusion:** due to the results of this study, type-2 diabetes disturbs the mitochondrial dynamics and the optimal function of skeletal muscles. On the other hand, exercise, as a beneficial and complication-free strategy has a essential role to maintenance the balance of mitochondrial dynamics and its optimal function in the diabetic skeletal muscles.

**Keywords:** aerobic exercise training , mitochondrial dynamics, diabetes, Rat

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## Introduction

All living organisms are dependent on the cellular and physiological mechanisms of homeostasis to maintain the function of the internal environment of the body. Mitochondria are the basis of cellular homeostasis through their multiple roles in energy production, biosynthesis, regulation of calcium and signaling, redox balance and production of reactive oxygen species(ROS)[1]. Mitochondria continually fluctuate in size and quantity through the two opposing processes of fusion and fission [2]. The distribution of mitochondrial DNA, calcium signaling, apoptosis, mitophagy, and the separation of mitochondria from daughter cells are all impacted by changes in mitochondrial shape and the interrelationships of the mitochondrial network [3, 4].

For cells to respond to external stimuli and environmental stress optimally, the fusion-fission balance must be precisely regulated [5]. As a result, variations in the fusion-fission equilibrium cause oxidative stress, mitochondrial malfunction, and metabolic abnormalities in the cell. The equilibrium of mitochondrial fusion and fission is strongly regulated by the proteins MFN1, MFN2, FIS1, and Drp1 [6]. The mitochondrial shape alterations are controlled at the molecular level by a dynamin-like GTPase. To fold mitochondrial membranes in a GTP-dependent way, dynamin-related protein 1 (Drp1) forms loop-like structures [3]. Fission protein 1 (FIS1), mitochondrial fission factor (MFF), mitochondrial dynamic proteins 49 (MiD49) and 51 kDa (MiD51), as well as mitochondrial dynamic protein 51 (MiD51), all recruit Drp1. However, mitochondrial outer membrane-resident mitofusin proteins 1 and 2 (MFN1,2) link two mitochondria by homo- and heterotypic dimerization [7].

Increasing evidence shows that mitochondrial dysfunction is associated with the different diseases including aging [8] neurodegenerative disorders [9], mitochondrial diseases [10], obesity , diabetes and cancer [11].

One of the diseases related to mitochondrial dysfunction is type 2 diabetes. Clinical complications of T2D include dyslipidemia, hyperglycemia [12], insulin resistance, and defects in insulin secretion from pancreatic beta cells [13]. The main cause of these complications is an increase in the production of ROS in mitochondria due to hyperglycemia [12, 14]. A common feature of mitochondrial morphology in T2D is increased mitochondrial fragmentation, which occurs through activation/up regulation of Drp1 or a decrease in the levels of MFN2. In addition, it was shown that impaired mitochondrial fusion is associated with insulin resistance in skeletal muscle [15] , glucose intolerance, and increased hepatic gluconeogenesis in liver-specific MFN2 knockout mice [16]. increased ROS generation, activation of JNK, and endoplasmic reticulum stress responses are caused by MFN2 deletion. Overexpressing the MFN2 protein increases insulin sensitivity and decreases lipid mediators

in the liver and skeletal muscle, according to studies in mouse models [17, 18].

Furthermore, it has been shown that mitochondrial fission is enlarged in T2D dyslipidemia models [19]. Increased mitochondrial fragmentation along with increased levels of mitochondrial Drp1 and FIS1 proteins have been observed in diabetic muscle cells[20]. The etiology of diabetic problems such as nephropathy [21], retinopathy [22], neuropathy, stroke, and cardiac ischemia [23] is significantly influenced by mitochondrial fission brought on by an increase in Drp1. Particularly, T2D is linked to a reduction in MFN2 expression, which affects mitochondrial fusion and reduces oxidative phosphorylation in skeletal muscle [24, 25]. When mitochondrial homeostasis is disrupted in skeletal muscle, a series of atrophic signals are activated that leading to the myofiber Destruction [26].

The previous studies have shown that exercise through the improvement of the mitochondrial metabolism, biogenesis and quality control prevent or delay the mitochondrial dysfunction. The exercise regulates the function of proteins that involved in regulation of mitochondrial dynamics through the post-translational modulation mechanisms [27]. Previous studies have shown that aerobic exercise training training leads to a 60–120% increase in mitochondrial protein content [28]. Citrate synthase activity mediates exercise-induced changes in the activity of fusion-related proteins. Also this to be mediated by two critical exercise-responsive and transcriptional co-activators PGC1α and estrogen-related receptor (ERRα), which drive mitochondrial biogenesis [29]. Notably, aerobic exercise training can increase expression of PGC-1α mRNA and protein to improve the level of MFN2 protein and promote mitochondrial fusion [30].However, the reduction in fission- and mitophagy-related proteins appears to be independent of changes in mitochondrial content. These changes may contribute to the improvements in insulin sensitivity and substrate utilization that are observed after exercise training [31]. On the other hand, Aerobic exercise training than the resistance or combined training improves the skeletal muscle mitochondrial dynamics. aerobic exercise training produces a larger, more fused mitochondrial tubular network in skeletal muscle. In contrast, fewer alterations or no changes in mitochondrial morphology and regulators of mitochondrial dynamics were observed following Combined training or resistance training. These changes lead to increased mitochondrial size, increased fusion, improved mitochondrial respiration, and improved insulin sensitivity. [32]. Despite of the doing various studies on exercise and mitochondrial function, the effect of exercise on proteins related to the mitochondrial dynamics in T2D has been less studied. Therefore, in the present study, the researchers seeks to find an answer to the questions of whether eight weeks of aerobic training has an effect on the levels of MFN1, MFN2, FIS1, and Drp1 proteins in the

gastrocnemius muscle tissue in male wistar diabetic rats.

## 1. Methods and Materials

### *1.1. Animals*

The present study is experimental type which has been approved by the ethics committee of the Islamic Azad University of Ahvaz branch. The 20 male Wistar rats with 8 weeks aged and body weight 200±20 g were placed in standard polypropylene cages (three rats per cage) under a light/dark cycle of 12 hours and an environment temperature of 22-25 Celsius degrees. The rats were randomly divided into four groups: Healthy Control(HC), Healthy Training[33], Diabetic Control(DC) and Diabetic Training [34].

**Induction of Diabetes:** Type 2 diabetes was induced according to the protocol of Zhang et al [35] and Liu et al [36]. Rats of the Healthy groups were fed by a standard diet with a total caloric volume of 20 kJ/kg (5% fat, 52% carbohydrates, 20% protein), while rats of the diabetic groups were fed by a high-fat and high-calorie diet with a total caloric volume of 40 kJ/kg (20% fat, 45% carbohydrate, 22% protein). All of the groups used their own diet for eight weeks. At the end of the fourth week, diabetic rats received an intraperitoneal injection of low-dose Streptozotocin in one step (30 mg/kg, dissolved in 0.1 M sodium citrate buffer at pH 4.4) from Sigma-Aldrich in St. Louis, Missouri. Blood glucose was tested using a glucometer (Accu-Chek Performa; Roche Diagnostics, USA) a week after the STZ injection. The rats which blood glucose level were less than 16.7 mmol/L or 300 mg/dL, received streptozotocin same as the previous step (25 mg/kg). The rats of the healthy groups were injected of the citrate buffer (0.25 ml/kg) simultaneously with diabetic groups. As previously mentioned, the diets were maintained until the end of the eighth week. The blood glucose level was assessed once more at the end of the eighth week, and rats with blood glucose levels more than 300 mg/dL were classified as diabetics.

**Aerobic exercise training :** After the induction of type 2 diabetes in the diabetic groups, the HT and DT groups performed aerobic exercise training for eight weeks based on the protocol of Rocha-Rodrigues et al [37]. Based on this protocol, in order to familiarize the animals with the treadmill, one week of training was done with an intensity of 10 m/min for 5 minutes with a frequency of 5 sessions per week. then, the training program for eight weeks and based on the gradual overload principle, increasing from 15 m/min for 30 minutes in the first week to 25 m/min for 60 minutes in the eighth week. To reach a steady state, all training variables were kept constant in the last two weeks. Also, to ensure the non-return of diabetes in diabetic groups, blood glucose concentration and body weight of the rats were measured at the end of each week. Table 1 shows a summary of the aerobic exercise training program.

**Tissue samples:** The intraperitoneal injection of a mixture of ketamine (50 mg/kg) and xylazine (4 mg/kg) was used to simultaneously sedate all of the rats in the four groups 48 hours after the final training session. Blood samples were taken from the heart. Blood was centrifuged for 15 minutes at 800× g at 4 °C to get the serum. The tissue of the gastrocnemius muscle was extracted by a specialist and washed in PBS. After that, liquid nitrogen was used to keep the removed muscle tissue at -80°C until the last test.

*1.2. Procedure*

**Measurement of lipid profile and insulin:** The serum lipid profile was determined on fully automated Olympus AU640 analyser (Olympus, Hamburg, Germany) using commercial assays. triglycerides (TG), High Density Lipoprotein (HDL), Low Density Lipoproteincholesterol (LDL) were measured using enzymatic methods and reagents from Diasys (Holzheim, Germany). Fasting insulin was tested with Insulin INS-Rat kit (Qayee-Bio, Shanghai, China) by ELISA.

**Western blot analysis:** Western blot method was used to measure the levels of proteins related to the mitochondrial dynamics. Muscle homogenates were prepared by grinding muscle tissue in ice-cold lysis buffer (Invitrogen) in the presence of protease inhibitor cocktail, 5 mM phenylmethylsulfonyl fluoride (Sigma), and Phos-STOP (Roche Applied Sciences, Indianapolis, IN). The homogenates were then centrifuged for 10 min at 14,000 x g. The resulting supernatant was decanted and the tissue lysates were stored at −80°c until the time of analysis. Protein concentrations were measured using a BCA protein assay kit (Pierce Biotechnology, Rockford, IL). 32 µg's (0.8 µg/mL) of muscle lysate were solubilized in Laemmli sample buffer containing 5% β-mercaptoethanol and boiled for 5 min. 40 µL's of sample was then loaded into 4–12% Tris Glycine gels (Novex) and separated via sodium dodecyl sulfate polyacrylamide gel electrophoresis at 125 volts for 1.5 hours (SDS-PAGE: Invitrogen).

The gels were transferred to polyvinylidene fluoride membranes (Bio-Rad), and blocked with 5% bovine serum albumin (BSA) in phosphate-buffered saline with 0.1% Tween-20 (PBST) for 1 hour. Membranes were then incubated overnight with anti-MFN1 (R & D Systems; Minneapolis, MN, catalog no. GTX17218), anti-MFN2 (Cell Signaling Technology; Danvers, MA, catalog no. ab50838), and anti-Drp1 (Abnova; Walnut, CA, catalog no. ab156951) and anti-FIS1 (Thermo Fisher Scientific; Waltham, MA, GTX111010) in 5% BSA solution. Membranes were washed with PBST and incubated with species-specific horseradish peroxidase-conjugated secondary antibodies (GAPDH; catalog no. ab181602) diluted 1:10,000 in 5% BSA solution. Immunoreactive proteins were visualized by enhanced chemiluminescence reagent (ECL Prime; GE Healthcare) and quantified by densitometric analysis using ImageJ software62.

All antibodies were internally validated prior to use. All bands displayed were subject to quantification.

*1.3. Statistic*

The mean and standard deviation were utilized to characterize the data in the current investigation. The Levin test was used to assess the homogeneity of variances, and the Shapiro-Wilk test was utilized to assess the normality of the data distribution. repeated actions Body weight and glucose levels at different weeks were compared within and between groups using an ANOVA analysis, and variables within groups were compared using a one-way ANOVA analysis with Tukey's post hoc. All data were analyzed at a significance level of 0.05 by SPSS-23 software.

## 2. Results

*2.1. Weight changes*

An analysis of variance with repeated measures was used to assess the weight changes in rats in the four groups since the weight of the rats was assessed at 17 distinct points (before the intervention and at the end of each week of the intervention) (Fig. 1a). The data analysis revealed that there was no Significant difference between groups in the weight of the rats at birth ($p = 0.251$). The average weight increases in the DC and DT groups were larger than their baseline weight at the end of the eighth week ($P = 0.014$). Additionally, as compared to the HC and HT groups, the average weight of the DC and DT groups increased considerably. There was no discernible difference between the initial weight and the weight at the end of the eighth week in the HC and HT groups ($p = 0.451$). In healthy groups, according to the rats were adults and received standard diet, no significant change in their weight was observed. The weight gain in DC group increased linearly until the 16th week, but a significant decrease was observed in the DT group that performed aerobic exercise training for eight weeks from the 9th to the 16th week ($p = 0.018$). Therefore, aerobic exercise training prevented the weight gain of rats in the DT group. Fig. 1a shows the weight changes of rats in four groups at the end of each week of the intervention.

*2.2. Changes in Blood Glucose levels*

Blood glucose levels rose from the start of the intervention in the high-fat groups (DC and DT) and continued to rise after the fourth week of streptozotocin administration. The blood glucose levels were higher than 300 mg/dL and continued to rise until the eighth week. The blood glucose level in the DT group started to drop in the ninth week, concurrent with the initiation of aerobic activity, and it did so in a manner that was noticeably different from the DC group until the end of the sixteenth week ($p = 0.001$). However, the DT group's blood glucose level remained over 300 mg/dL. In the HC and HT groups, during the entire of intervention period, blood glucose levels were not significantly changed compared to its

primary level. Fig. 1b shows the changes of the blood glucose levels in four groups at 17 different weeks.

### *2.3. The changes of in the proteins related to the mitochondrial dynamics in diabetes*

to compare the MFN1, MFN2, FIS1 and Drp1 proteins of gastrocnemius muscle in four groups, one-way analysis of variance (ANOVA) with Tukey's post hoc test was used. Data analysis with ANOVA showed that significant differences were observed between groups in all of the MFN1, MFN2, FIS1 and Drp proteins. Tukey's post hoc analysis revealed that the levels of proteins associated with mitochondrial fission (MFN1 and MFN2) in the gastrocnemius muscle were substantially lower in the DC group compared to the HC group (P=0.025,F=32.93 and P=0.019, F=27.38 Respectively). This indicates that the MFN1 and MFN2 proteins in the gastrocnemius muscle tissue decreased as a result of the induction of diabetes. Additionally, the DC group had substantially greater levels of the proteins (FIS1 and Drp1) linked to mitochondrial fission proteins than the HC group (P=0.001, F=70.87, Effect Size=0.82 and P=0.001, F=43.89,Effect Size=0.79 Respectively). This means that the induced diabetes led to an increase in FIS1 and Drp1 proteins in the gastrocnemius muscle tissue (Fig.2a,b,c,d).

### *2.4. The effect of aerobic exercise training on levels of proteins that related to the mitochondrial dynamics*

Tukey's post hoc analysis revealed that there was a significant rise in the levels of MFN1 and MFN2 proteins in the DT group that engaged in aerobic activity for eight weeks as compared to the DC group (P=0.027, P=0.005, Effect Size= 0.76 Respectively). It indicates that the levels of proteins associated with mitochondrial fusion in the muscle tissue of diabetic rats significantly increased as a result of aerobic exercise training . However, the analysis of the HC and HT groups revealed that the eight weeks of aerobic exercise training in healthy rats did not result in any appreciable changes in the levels of MFN1 and MFN2 proteins. Additionally, after eight weeks of aerobic activity, the levels of the proteins FIS1 and Drp1 in the muscle tissue of the DT group were significantly lower than those of the DC group (P=0.031, P=0.012, Effect Size=0.81 Respectively). This means that aerobic training led to a significant reduction of proteins that related to the mitochondrial fission in the muscle tissue of diabetic rats. However, the comparison of the HC and HT groups showed that eight weeks of aerobic exercise training s did not lead to a significant change in the levels of FIS1 and Drp1 proteins in healthy rats (Fig.2a,b,c,d).

Also Western blot analysis of MFN1, MFN2, DRP1 and FIS1 proteins to GAPDH ratio in the muscle tissue of four groups is shown in figure 3. As shown in Fig. 3, we found that in DC

group, the MFN1 and MFN2 proteins expression decreased, while the levels of Drp1 and FIS1 proteins increased (P<0.05, Effect Size=0.85). in the DT group that performed aerobic exercise training compared to the DC group, the levels of proteins MFN1 and MFN2 increased, but the Drp1 and FIS1 proteins levels were decreased (P<0.05, Effect Size=0.74). Also, no significant difference was observed between groups HC and HT in any of the measured proteins (P>0.05, Effect Size=0.86).

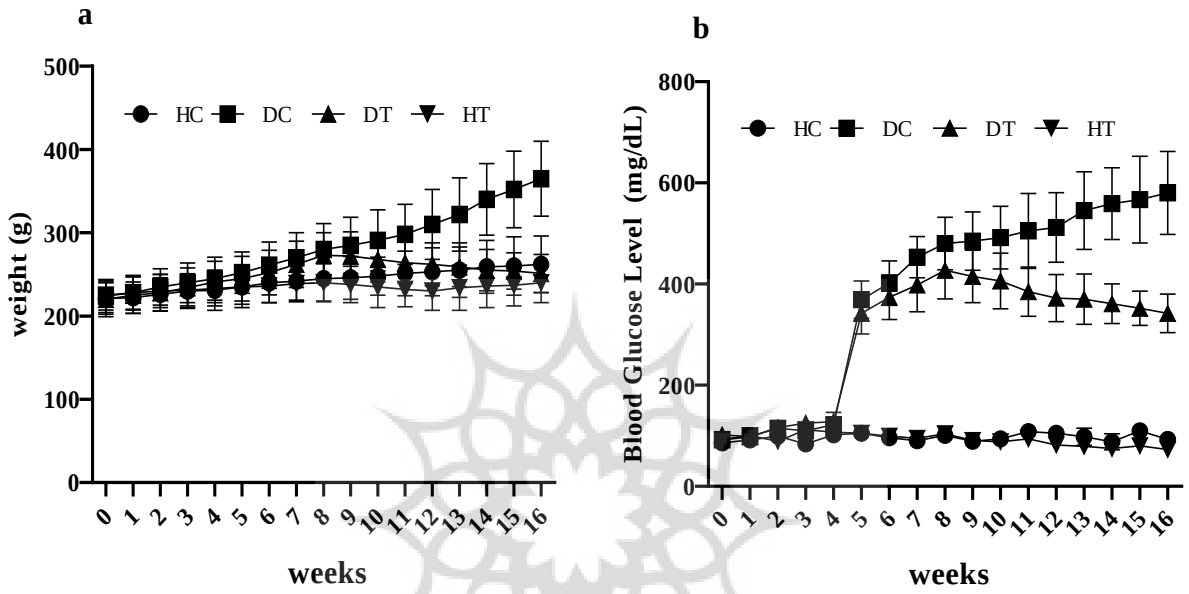


**Figure 1.** The changes of weight (a) and blood glucose levels (b) of groups in 17 weeks

**Table 1:** Aerobic exercise training program

| Week | familiarization | 1th | 2th | 3th | 4th | 5th | 6th | 7th | 8th |
|---|---|---|---|---|---|---|---|---|---|
| Intensity(m/min) | 10 | 15 | 16 | 18 | 20 | 21 | 23 | 25 | 25 |
| Duration [29] | 5 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 60 |
| Frequency (per week) | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |

**Lipid profile changes:** the Table-2 shows the changes of lipid profile including Cholesterol, Triglycerides (TG), HDL, LDL and Insulin in groups. As shown in the table, the levels of TG, Cholesterol, LDL and Insulin levels increased in DC group compared to the HC group. However, the levels of HDL in DC group has decreased than the HC group. Also, in the groups that did aerobic exercise training compared to their control groups, the levels of TG, Cholesterol, LDL and Insulin decreased, but an increase in HDL levels was observed.

**Table-2:** Lipid profile changes in groups

| Groups | HC | DC | HT | DT |
|---|---|---|---|---|
| Chol (mg/dL) | 66.30±11.29 | 134.00±15.10 | 62.37±12.05 | 108.60±17.41 |
| TG (mg/dL) | 75.33±13.05 | 185.30±21.06 | 70.40±15.32 | 155.64±18.29 |

| | | | | |
|---|---|---|---|---|
| HDL (mg/dL) | 6.37±1.42 | 2.17±0.76 | 7.14±1.94 | 4.15±1.38 |
| LDL (mg/dL) | 37.50±4.05 | 86.73±4.71 | 35.22±5.19 | 80.35±6.67 |
| INSULIN (mU/L) | 11.26±0.76 | 15.70±0.53 | 10.47±0.91 | 14.82±1.20 |

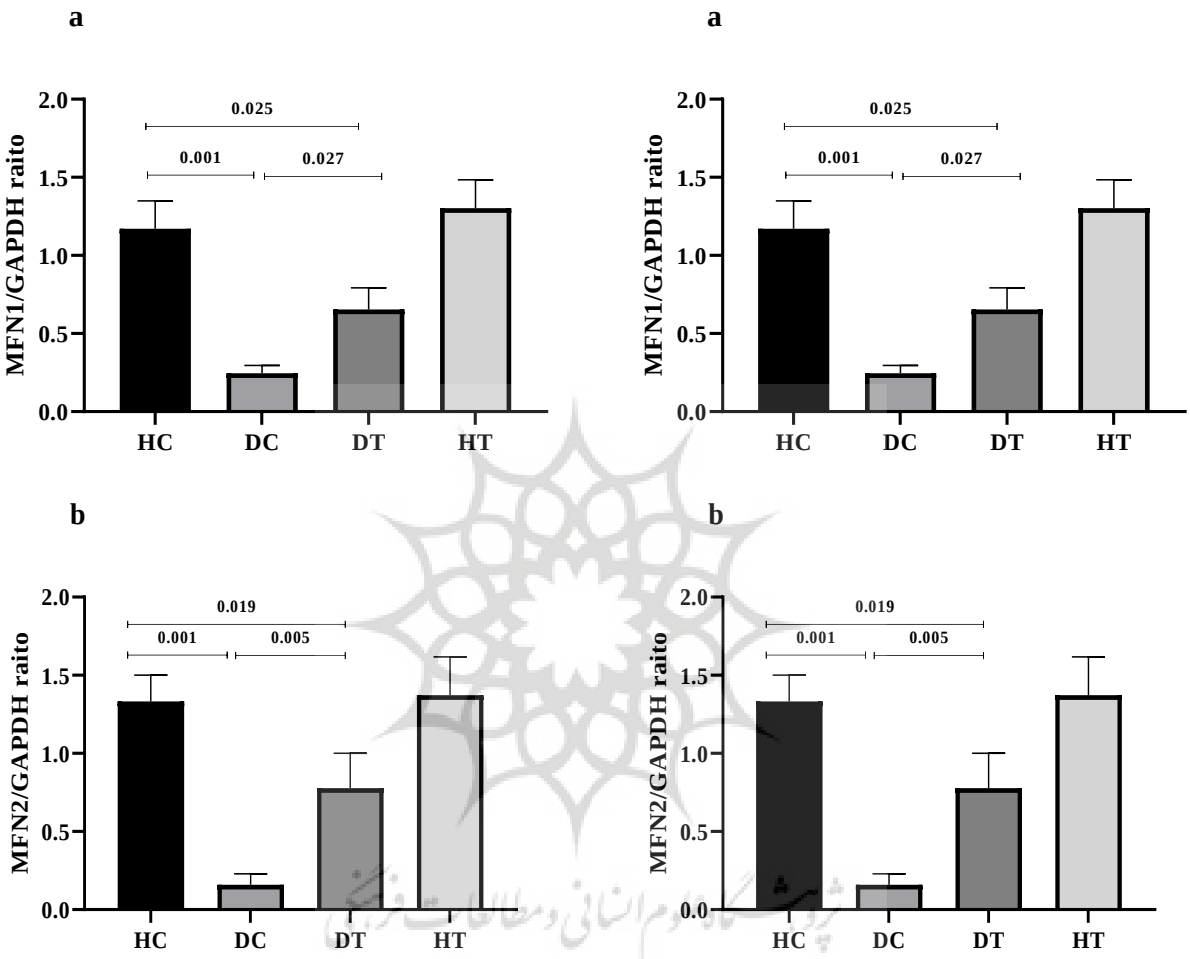


**Figure 2:** The changes of in the mitochondrial dynamic-related proteins in diabetes and exercise.

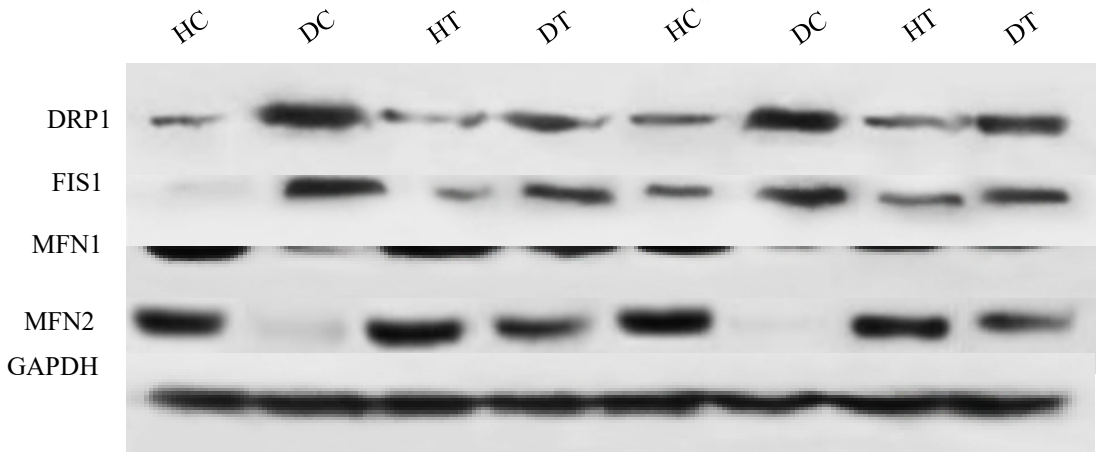

**Figure 3.** Representative bands for each group using GAPDH as load control.

## 3. Discussion

According to earlier research in humans [25] and animal models [38, 39] type 2 diabetes and insulin resistance are strongly connected to a rise in mitochondrial fission-related proteins and a decrease in fusion-related protein levels. The excessive release of mitochondrial H2O2, which is thought to be the main source of malfunction in mitochondrial dynamics and results in the disruption of the insulin signaling pathway [40], is likely responsible for the imbalance between mitochondrial fusion and fission in obesity and type 2 diabetes. Lin et al. [41] showed that mitochondrial ROS generation mediates the association between mitochondrial dynamics and insulin resistance. Fealy et al [42] illustrated that excessive ROS production stimulates the secretion of Drp1 to move to the mitochondrial membrane and, as a result, activate mitochondrial fission. This stimulation of Drp1 is responsible for reducing the membrane potential and Facilitates the entry of ROS into the cytosol, and as a result, it leads to disruption of the insulin signaling pathway.

Heo et al [40] explained that the moderate aerobic exercise training increased mitochondrial fusion-related proteins such as MFN1, MFN2, and OPA-1 and decreased fission-related proteins such as FIS1 and Drp1. These changes in the mitochondrial dynamics-related proteins due to aerobic exercise training have been attributed to the reduction of H2O2 release from the mitochondria following these exercises.

It was shown in the current study that the levels of MFN1 and MFN2 proteins in the muscle tissue of the DC group were considerably lower than those in the HC group, but the levels of these proteins dramatically increased in the DT group that underwent eight weeks of aerobic exercise training. the main Mechanism for the decrease in the levels of MFN1 and MFN2 proteins in diabetes is attributed to the increased the production of ROS caused by hyperglycemia [12, 14]. MFN2 knockout animals exhibit increased hepatic gluconeogenesis and glucose intolerance, which have both been linked to defective mitochondrial fusion and enhanced insulin resistance in skeletal muscle [16, 18]. The decrease in the MFN1 and MFN2 proteins leads to the reduction activity of complex 1 and complex 4 and consequently decrease the exercise performance, because of the disorder in these two complexes are associated with the exercise intolerance and reduction the exercise performance [43]. Perry et al [44] in their study showed that the seven sessions of high-intensity interval training gradually increased the protein content of MFN1 and FIS1. Also, Ding et al [45]

reported that a session of treadmill running in rats increased MFN1 and MFN2 mRNA for 24 hours after exercise. Other studies have revealed that the transcription of MFN1 and MFN2 is regulated by PGC-1α through the estrogen related receptor-α (ERRα) [29].

regular physical activity as a strong physiological stimulus, can creates the mitochondrial adaptations that counteracts the adverse effects of diabetes on skeletal muscle mitochondria [46]. Among the main mechanisms which exercise through them can reduce the mitochondrial disorders caused by diabetes, can be mentioned the increase of the important proteins levels that involved in mitochondrial function such as PPARg, PGC-1α, HSPs and UCP3 [47].

Another finding of the present study was the significant increase of proteins that related to the mitochondrial fission Including FIS1 and Drp1 in diabetes and significant decrease of these proteins due to the eight weeks of aerobic exercise training.

Disruption of mitochondrial dynamics has been exposed in the many of diseases, especially the type 2 diabetes [48]. The increase in the Drp1 protein due to the diabetes, may lead to a decrease in membrane potential through the sensitizing of Mitochondrial Permeability Transition Pore (mPTP) and consequently increasing the production of ROS [49]. Previous studies have demonstrated that physical activities reduce the mitochondrial fission in diabetic and insulin-resistant muscles and lead to the improvement of fatty acid oxidation and increase in the insulin sensitivity [50]. Also, Consonant with the results of the present study, Axelrod et al [31] confirmed that the twelve weeks of aerobic training significantly reduces the muscle FIS1 levels. Furthermore, increased fusion following exercise training promotes increased connectivity of the mitochondrial reticulum and is associated with improved metabolism and mitochondrial function.

Finally, it has been proved that physical activities through the increase of fusion-related proteins such as MFN1, MFN2 and OPA-1 and decrease the fission-related proteins such as Drp1 and FIS1 play an important role in improvement of the metabolism and glucose absorption in skeletal muscles and as a result the improvement of insulin resistance in type 2 diabetes[51]. Considering that in this study, diabetes was induced by a high-fat diet and STZ injection, and since the important role of the nutrition in controlling the diabetes, it is recommended that future researchers to assess the changes in mitochondrial dynamics study the herbal nutritional supplements along with the exercise. Another limitation of the present study was the lack of histological evaluation of muscle. It is suggested that in future research, muscle histology should also be considered while examining proteins related to mitochondrial dynamics.

## 4. Conclusions

When mitochondrial homeostasis is disturbed in skeletal muscle, an array of atrophic signals become activated that induce myofiber degradation. Imbalances in fusion and fission activity are one example of this dyshomeostasis, as seen in certain human myopathies such as diabetes type 2.

due to the important role of mitochondria in energy production, antioxidant defense and even cognitive functions of the body, the optimal functional of this organelle is essential. on the other hand, exercise can be considered as a useful and non-pharmacological strategy through the creating of the balance between mitochondria fusion-fission and as a result the homeostasis of mitochondrial dynamic.

## Conflict of interest

The authors declared no conflicts of interest.

## Authors' contributions

All authors contributed to the original idea, study design.

## Acknowledgment

We want to thank all the participants in this study for their time and willingness to share their experiences. Their contributions have been invaluable in helping us to understand the topic and draw meaningful conclusions.

## Ethical considerations

The author has completely considered ethical issues, including informed consent, plagiarism, data fabrication, misconduct, and/or falsification, double publication and/or redundancy, submission, etc. The current study has been approved by the ethics of committee of Islamic Azad University Ahvaz branch with ID number of IR.IAU.AHVAZ.REC.1401.202.

## Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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